Expired Breath Hydrogen Peroxide Is a Marker of Acute Airway Inflammation in Pediatric Patients with Asthma

ANN WANG DOHLMAN, HUGH R. BLACK, and JAMES A. ROYALL

Department of Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama

Airway inflammation is important in the development and progression of many pulmonary disorders, including asthma. We hypothesized that the hydrogen peroxide (H_2O_2) concentration in expired breath may be a marker of airway inflammation. Expired breath condensate was collected by cooling and the H_2O_2 concentration was measured fluorimetrically. Thirty-five samples were collected from 22 pediatric patients with asthma who were 7 to 18 yr of age and from 11 healthy, nonasthmatic controls. Asthmatic subjects were determined to be well or sick (acute disease of the upper or lower respiratory tract) by clinical examination. Pulmonary function tests were determined to be abnormal if there was a > 15% reduction in FEV_1 or > 20% reduction in FEF_{25-75} compared with baseline values. Expired breath H_2O_2 was elevated in asthmatic subjects compared with controls (0.81 ± 0.70 versus 0.25 ± 0.27 μmol/L). The difference was primarily due to elevation of H_2O_2 in sick asthmatic subjects, whose expired breath H_2O_2 level of 1.5 ± 0.5 (n = 10) was different from that of well asthmatics (0.54 ± 0.56, n = 25). There was a high correlation between expired breath H_2O_2 and clinical status. Elevation of expired H_2O_2 occurred with either acute upper or lower respiratory tract disease. There was no statistically significant correlation between expired breath H_2O_2 level and pulmonary function test results. We conclude that elevation of H_2O_2 in the expired breath condensate is a simple, noninvasive method that can be used as a biochemical marker of airway inflammation. **Dohlman AW, Black HR, Royall JA. Expired breath hydrogen peroxide is a marker of acute airway inflammation in pediatric patients with asthma. Am Rev Respir Dis 1993; 148:955–60.**

Activation of inflammatory cells, including neutrophils, eosinophils, and macrophages, induces a respiratory burst resulting in marked production of superoxide anion (O_2^-). This oxidant species undergoes spontaneous or enzyme-catalyzed dismutation to form hydrogen peroxide (H_2O_2). Thus, H_2O_2 levels reflect both divalent reduction of oxygen and the dismutation of O_2^-. Production of H_2O_2 has been specifically used to quantify the respiratory burst of neutrophils (1). H_2O_2 appears to be an important reactive oxygen species causing cellular injury, perhaps via further reactions leading to more reactive species, such as hydroxyl radical and lipid peroxidation products. Evidence of reactive oxygen species–mediated tissue injury has been noted in a variety of immune-mediated disorders. Additionally, oxidants themselves may serve as mediators that amplify the immune response (2, 3). Two reports have demonstrated increased H_2O_2 in the expired breath of intubated adult patients with adult respiratory distress syndrome, an acute diffuse inflammatory disorder of the lung leading to respiratory failure (4, 5).

Asthma is a chronic lung disorder with the cardinal features of reversible airway obstruction, airway inflammation, and airway hyperresponsiveness. Increased airway inflammation may be present chronically or during acute exacerbations in mild to severe asthma. The development of airway inflammation and hyperresponsiveness in asthma is a complex process. Techniques to monitor airway inflammation are needed to delineate the role of airway inflammation in the development of asthma, the progression of asthma, and the effect of anti-inflammatory therapy on this progression. Recent studies, many utilizing fiberoptic bronchoscopy with bronchoalveolar lavage or endobronchial biopsy to obtain lower airway samples, have identified inflammatory cells and mediators as central participants in the pathogenesis of asthma (6–8). These relatively invasive techniques have been applied in adults. However, in the pediatric population, in whom asthma and other pulmonary disorders frequently have their onset, investigatory techniques should be simple and noninvasive.

We hypothesized that expired breath H_2O_2 concentration may serve as a simple, noninvasive method to document airway inflammation. In this study, we have quantified H_2O_2 in the expired breath condensate from a group of pediatric patients with asthma followed at our institution. In addition, we compared the expired breath H_2O_2 results with the patients' clinical status and pulmonary function tests at the time of sample collection.

METHODS

Subjects

Asthmatic subjects between the ages of six and 18 yr were recruited from patients who received regular care at the Pediatric Allergy Clinic of The

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Correspondence and requests for reprints should be addressed to James A. Royall, M.D., Division of Critical Care and Division of Pulmonology, The Children's Hospital of Alabama, ACC 504, 1600 7th Avenue South, Birmingham, AL 35233.

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Children's Hospital of Alabama. All subjects benefitted from the use of at least one daily prescribed asthma medication. All asthmatic subjects had at least 1 yr of routine clinical evaluation and routine pulmonary function testing. They were known to perform pulmonary function testing reliably, and baseline pulmonary function values had been previously determined. Therapy was adjusted by the patients' physician as appropriate for their clinical status. Control subjects were healthy, nonsmoking individuals with no known or suspected acute or chronic medical conditions. This study was approved by the Institutional Review Board of the University of Alabama at Birmingham. Informed consent was obtained from subjects 14 yr of age or older, and consent was obtained from a parent or guardian of younger patients.

Clinical Examination, Pulmonary Function Testing, and Collection of Expired Breath Condensate

On enrollment in the study, a history was obtained and physical examination was performed by the investigator who was the asthmatic subjects' usual physician (A.W.D.). At the time of this examination, this investigator recorded whether the patient was in their usual state of health (well) or whether there was clinical evidence of acute respiratory disease (sick). Additionally, this investigator recorded a clinical impression of upper airway disease or lower airway disease as the primary symptomatology. Acute upper respiratory illness was defined as a history of the acute onset of headache, rhinorrhea, and/or sore throat still present at the time of the examination and the presence of mucosal erythema and/or swelling with or without purulent drainage on physical examination. Acute lower respiratory illness was defined as a history of acute onset of coughing or wheezing still present at the time of the examination and evidence of airway obstruction by physical examination. Routine spirometry was performed (Multispiro Sensor; Multispiro Inc., Aliso Vejo, CA) and was assessed to be decreased from the previously determined baseline study if there was a reduction in FEV_1 of > 15% or a reduction in FEF_{25-75} of > 20% (9).

Following clinical evaluation and pulmonary function testing, expired breath condensate was collected in a manner similar to that described by Baldwin and colleagues (4) and Sznajder and colleagues (5). At least 3 ml of expired breath condensate was collected on ice by expiration through an ice-cooled tube (figure 1). This ensured minimal evaporative loss of H_2O_2. The subject breathed at a comfortable frequency and tidal volume and expired into a mouthpiece, which also served as a saliva trap. Expired breath condensate collection usually took 10 to 15 min. The collection system was washed and dried between each sample collection.

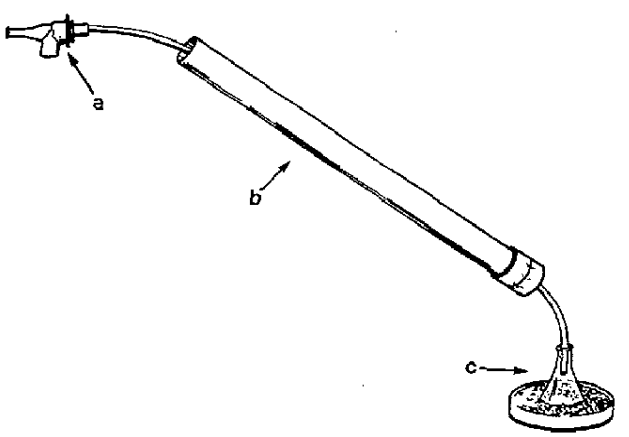

Figure 1. System for collection of expired breath condensate. Subjects expired into a mouthpiece (a) adapted from an aerosol drug delivery system (Inspirease®; Schering Corp., Kenilworth, NJ), which also served as a saliva trap. This was connected to tubing, which passed through a section of polyvinylchlorine pipe packed with ice (*b*). The pipe was placed in a stand at about ∠45° to prevent pooling of condensate. The condensate was then collected in an ice-cooled flask (*c*).

There was no detectable H_2O_2 in the water that had been placed in the tubing after patient collection and washing.

Quantification of Expired Breath Condensate H_2O_2

The concentration of H_2O_2 in expired breath condensate was quantified with a fluorimetric assay based on H_2O_2 reaction with horseradish peroxidase to form compound I, which then oxidizes p-hydroxyphenylacetic acid to form a fluorescent product (1). Excitation and emission scans were performed on the oxidized product (Fluorometer model LS-50; Perkin-Elmer Corp., Norwalk, CT), and a peak excitation wavelength of 295 nm and peak emission wavelength of 405 nm were determined. Stability of the fluorescent product at −20° C was documented by performing serial standard curves on a range of H_2O_2 concentrations from 0 to 10 μM in water stored at −20° C and finding similar values of slope and y-intercept. Additionally, the fluorescent product from the addition of reagent H_2O_2 to expired breath condensate was stable after storage at −20° C.

Immediately after collection of the expired breath condensate, 1.5 mM p-hydroxyphenylacetic acid (Sigma Chemical Co., St. Louis, MO) and 100 μg/ml horseradish peroxidase (Sigma) were added, and the condensate was frozen at −20° C. Quantification of H_2O_2 on groups of samples was performed within 1 month of collection. For each group of determinations a standard curve was performed. The specificity of the fluorescent signal for H_2O_2 was demonstrated by reduced fluorescence in the presence of catalase. Expired breath condensate was collected from five asthmatic subjects and divided into two aliquots. An addition of 300 U/ml of catalase (Worthington Biochemical Corp., Freehold, NJ) was made to one aliquot prior to performing the assay as described above. Four of the samples had an H_2O_2 concentration of 0.21 to 0.40 μmol/L, and the prior addition of catalase resulted in no detectable H_2O_2. One sample had an H_2O_2 level of 1.1 μmol/L that was reduced to 0.17 μmol/L by prior catalase addition.

Statistical Analysis

Results are given the mean ± SD. Statistical analysis was performed using two statistical software packages (Statgraphics 3.0; Rockville, MD and Primer; McGraw-Hill, New York, NY). A two-tailed *t* test or a two-tailed Fisher's exact test, with $p < 0.05$ considered significant, were used when appropriate.

RESULTS

On a group of 22 asthmatic subjects, 35 collections of expired breath condensate, clinical examination, and pulmonary function testing were performed (table 1). Two evaluations were performed on 11 subjects, and three evaluations were performed on one subject. There were 18 male and four female asthmatic subjects from 7 to 18 yr of age (mean 13.2 ± 3.0 yr). All 22 asthmatic subjects were taking at least one inhaled or oral β-adrenergic agent daily or as needed, and 12 were taking routine oral theophylline. Eleven of the asthmatic subjects were receiving daily anti-inflammatory medication. Seven used daily inhaled cromolyn and/or inhaled corticosteroids alone, one used alternate-day oral corticosteroids alone, two used both daily inhaled anti-inflammatory medication and alternate-day oral corticosteroids, and one used both daily inhaled anti-inflammatory medication and daily oral corticosteroids. The control group consisted of 11 healthy, nonsmoking males (n = 7) and females (n = 4) from 7 to 39 yr of age (mean 24.0 ± 6.6 yr).

Clinical Examination and Pulmonary Function Tests

The results of clinical examination and pulmonary function testing are summarized in table 2. Of the 35 evaluations, 10 examinations resulted in a finding of the patient having acute respiratory illness. Six patients were assessed as having upper respiratory tract illness (three with rhinosinusitis, two with pharyngitis, and one with suspected viral upper respiratory infection). For five of these six patients, there was no change from the baseline pulmonary function tests. Only the patient with a suspected viral upper respiratory infection (number 20) had a significant decrease in

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TABLE 1

CHARACTERISTICS OF ASTHMATIC SUBJECTS

Subject No.	Age (yr)/Sex	CS/Crom	BD	Recent CS Sample 1	Sample 2	Sample 3
1	13/M		X	Yes		
2	17/F		X	No		
3	11/M	X	X	Yes	Yes	
4	18/M	X	X	No		
5	12/M	X	X	No	Yes	
6	14/M		X	No	No	No
7	7/M	X	X	No	No	
8	13/M		X	Yes		
9	9/M	X	X	Yes	Yes	
10	11/M	X	X	No	No	
11	18/M	X	X	No	No	
12	10/F	X	X	No		
13	15/M		X	No	No	
14	7/F	X	X	No		
15	13/M	X	X	No	No	
16	16/F		X	No		
17	11/M	X	X	No	No	
18	14/M		X	No		
19	13/M		X	Yes		
20	15/M		X	No	No	
21	15/M		X	No		
22	17/M		X	No	No	

Definition of abbreviations: CS/Crom = routine use of corticosteroid or cromolyn therapy; BD = daily use of bronchodilator therapy; Recent CS = acute use of oral or intravenous corticosteroid therapy within 2 wk of evaluation.

TABLE 2

CLINICAL EXAMINATION AND PULMONARY FUNCTION TEST RESULTS FOR ASTHMATIC SUBJECTS

Subject No.	Clinical Examintion Sample 1	Sample 2	Sample 3	Pulmonary Function Test Sample 1	Sample 2	Sample 3
1	Well			No Δ		
2	Well			↓FEV_1 and FEF_{25-75}		
3	Sick (L)	Well		↓FEF_{25-75}	↓FEF_{25-75}*	
4	Sick (L)			↓FEF_{25-75}		
5	Well	Well		No Δ	↓FEF_{25-75}*	
6	Well	Well	Well	↓FEF_{25-75}	↓FEF_{25-75}	↓FEF_{25-75}
7	Well	Well		No Δ	No Δ	
8	Well			No Δ		
9	Well	Sick (U)		↓FEF_{25-75}*	No Δ	
10	Well	Sick (U)		↓FEV_1 and FEF_{25-75}*	No Δ	
11	Well	Well		No Δ	No Δ	
12	Well			No Δ		
13	Well	Well		No Δ	No Δ	
14	Sick (U)			No Δ		
15	Sick (U)	Well		No Δ	No Δ	
16	Sick (U)			No Δ		
17	Sick (L)	Well		↓FEV_1 and FEF_{25-75}	No Δ	
18	Well			No Δ		
19	Well			↓FEV_1 and FEF_{25-75}*		
20	Sick (U)	Well		↓FEV_1 and FEF_{25-75}	No Δ	
21	Well			No Δ		
22	Well	Sick (L)		No Δ	↓FEV_1 and FEF_{25-75}	

Definition of abbreviations: No Δ = no change from baseline; L = lower airway disease; U = upper airway disease.

* Acute wheezing within 1 mo of evaluation.

pulmonary function test values. Four patients were assessed as having acute lower respiratory tract disease, and in all of these cases there was a deterioration from the patient's baseline pulmonary function test values. In 25 of the evaluations, the patient was found to have no clinical evidence of acute disease. Of these 25, 16 had no deterioration from baseline and nine had decreased pulmonary function test values. Of the nine patients with decreased pulmonary function, despite a clinical examination indicating no acute respiratory disease, five had had an acute episode of airway obstruction requiring a change in therapy within the month (range 1 day to 1 month) prior to evaluation. One adolescent patient (number 6) accounted for three evaluations showing a decrease in pulmonary function with no evidence of acute disease and no recent history of acute wheezing.

Expired Breath Condensate H_2O_2 Concentration

There was no apparent relationship between age and H_2O_2 concentration in the expired breath condensate. The results of H_2O_2 concentration measurements from controls and asthmatic subjects are given in figure 2. The concentration in controls ranged from 0 to 0.8 μmol/L with a mean of 0.25 ± 0.27 μmol/L. The expired breath condensate H_2O_2 concentration in asthmatic subjects was 0.1 to 2.5 μmol/L with a mean of 0.81 ± 0.70 μmol/L. A comparison using a two-tailed *t* test indicated a significant difference ($p = 0.013$) in the concentration of H_2O_2 between the two groups. Based on these results, an H_2O_2 concentration of ⩾ 1.0 μmol/L was considered elevated for further comparisons.

In the asthmatic subjects, there was a high degree of correlation between clinical evidence of acute respiratory tract disease and an elevated expired breath condensate H_2O_2 concentration (table 3). The mean concentration of H_2O_2 in asthmatics who were determined to be clinically well ($n = 25$ evaluations) was 0.54 ± 0.56 μmol/L, which was significantly different ($p < 0.001$) from the mean H_2O_2 concentration of 1.5 ± 0.50 μmol/L ($n = 10$) in asth-

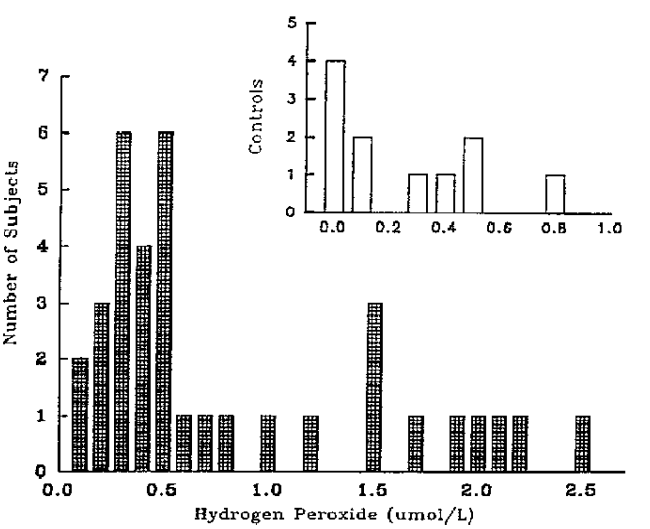

Figure 2. H_2O_2 concentration in expired breath condensate of asthmatic subjects and nonasthmatic controls. Results of the 35 samples from asthmatic subjects are shown in the lower graph (*shaded bars*). Results from the 11 control samples are shown in the upper graph (*open bars*). Control H_2O_2 concentrations ranged from 0 to 0.8 μmol/L (mean 0.25 ± 0.27 μmol/L), and those of asthmatic subjects ranged from 0.1 to 2.5 μmol/L (mean 0.81 ± 0.70 μmol/L). Analysis by a two-tailed *t* test indicated a significant difference between controls and asthmatic subjects ($p = 0.013$).

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TABLE 3

ASSOCIATION OF EXPIRED BREATH H_2O_2 CONCENTRATION WITH CLINICAL STATUS IN ASTHMATIC SUBJECTS*

H_2O_2 Concentration (μmol/L)	Clinical Status (subject no.)†	
	Well	Sick
< 1.0	1, 2, 3b, 5a, 6a, 6c, 7a, 7b, 8, 9a, 10a, 11a, 11b, 12, 13a, 13b, 15b, 17b, 18, 19, 20b, 21, 22a	22b
⩾ 1.0	5b, 6b	3a, 4, 9b, 10b, 14, 15a, 16, 17a, 20a

* $p \approx 7\ E^{-4}$ by two-tailed Fisher's exact test.

† For subjects having more than one H_2O_2 measurement, subject number is followed by letter indicating the temporal relationship of repeated samples.

matics determined to be clinically sick. Of the acutely sick asthmatics, there was no difference in the expired breath H_2O_2 concentration between those designated as having upper respiratory tract disease (1.6 ± 0.5 μmol/L, n = 6) and those designated as having lower respiratory tract disease (1.4 ± 0.6 μmol/L, n = 4).

The correlation between pulmonary function test results that showed a significant deterioration from baseline and presence of an elevated concentration of H_2O_2 in the expired breath condensate was not statistically significant (table 4).

The use of anti-inflammatory agents was a potential confounding factor in the use of expired breath condensate H_2O_2 as a marker of airway inflammation. Eleven of the 22 asthmatic subjects were receiving daily or alternate-day therapy with an anti-inflammatory medication. Of these 11 subjects, clinical evidence of either upper or lower acute airway disease was noted in seven, and in all cases the expired breath condensate H_2O_2 was elevated to > 1.0 μmol/L (range 1.2 to 2.2 μmol/L). Of the 35 evaluations, there were eight in which there had been acute use of oral corticosteroid within 2 wk preceding the evaluation. Of these eight evaluations, clinical examination revealed no evidence of acute airway disease in six, the expired breath H_2O_2 was < 1.0 μmol/L in five, and was > 1.0 μmol/L in only one. In two evaluations the subjects had clinical evidence of acute airway disease, and in both the H_2O_2 concentrations were > 1.0 μmol/L (1.5 and 1.7 μmol/L). These results indicate that if a subject had sufficient acute inflammation to result in symptomatology, despite the therapeutic use of anti-inflammatory agents, there would be a concomitant increase in expired breath condensate H_2O_2.

DISCUSSION

Airway inflammation appears to have a central pathogenic role in the initiation, progression, and acute exacerbations that occur in asthmatic patients (6). Airway inflammation has been documented in asthmatic patients by endobronchial biopsy (10, 11), by determination of respiratory membrane permeability from the protein content of bronchoalveolar lavage (BAL) fluid (12), and by identification of inflammatory cells and mediators in BAL fluid (6–8). A simple, noninvasive technique to quantify biochemical markers of airway inflammation could be a valuable tool for investigation of the role of airway inflammation in asthma and other pulmonary disorders. This may be especially true in pediatric patients, in whom relatively invasive techniques such as BAL and endobronchial biopsy are less likely to be used routinely.

The activation of inflammatory cells, including eosinophils, results in a respiratory burst and production of O_2^-, which undergoes enzyme-catalyzed or spontaneous dismutation to H_2O_2. Whether these reactive oxygen species have pathologic effects and whether increased levels are detectable depends not only on the amount of the species produced but also on further reactions that may consume the species. A group of oxidant scavengers—enzymatic scavengers, such as superoxide dismutase, catalase, and the glutathione peroxidase–reductase system, as well as nonenzymatic scavengers, such as vitamin E, urate, glutathione, and albumin—serve to protect against pathologic effects from reactive oxygen species (2, 13). When these endogenous antioxidant defenses are overcome, reactive oxygen species may react with biologically important molecules to produce tissue injury or they may escape the immediate site of production to be detectable in other compartments. Because H_2O_2 is uncharged and less reactive than other reactive oxygen species, H_2O_2 has the propensity to cross biological membranes and enter other compartments (2). Furthermore, because it is volatile, increased H_2O_2 in the airway equilibrates with air (14). Compared with the cellular antioxidant scavenging systems, the extracellular space and airway have significantly less ability to scavenge reactive oxygen species (13).

TABLE 4

ASSOCIATION OF EXPIRED BREATH H_2O_2 CONCENTRATION WITH PULMONARY FUNCTION IN ASTHMATIC SUBJECTS*

H_2O_2 Concentration (μmol/L)	Pulmonary Function Compared with Baseline (subject no.)†	
	Unchanged	Decreased
< 1.0	1, 5a, 7a, 7b, 8, 11a, 11b, 12, 13a, 13b, 15a, 17b, 18, 20b 21, 22a	2, 3b, 6a, 6c, 9a, 10a, 19, 22b
⩾ 1.0	9b, 10b, 14, 15ab, 16	3a, 4, 5b, 6b, 17a, 20a

* $p = 0.28$ by two-tailed Fisher's exact test.

† For subjects having more than one H_2O_2 measurement, subject number is followed by letter indicating the temporal relationship of repeated samples.

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Thus, the airway inflammation that occurs in asthma and other acute inflammatory processes involving the respiratory tract may be particularly likely to produce increased oxidant production that is detectable by increased H_2O_2 in expired breath.

In this study we have demonstrated a correlation between an elevation in the H_2O_2 concentration of expired breath condensate and acute respiratory disease in a group of asthmatic patients. The determination, based on history and clinical examination, of whether patients had an acute respiratory illness (sick) or were in their usual state of health (well) was made prior to pulmonary function testing and collection of the expired breath condensate. The high degree of correlation between the clinical respiratory status and the expired breath condensate concentration of H_2O_2 indicates that the significant airway inflammation that mediates clinical symptomatology also leads to detectable increases in reactive oxygen species, which are important participants in the inflammatory process. The accuracy of the clinical impression of one investigator (A.W.D.) as to whether patients had acute upper versus lower airway disease is supported by the pulmonary function test results. All of the asthmatic subjects with clinical acute lower airway disease had a decrease in pulmonary function test values compared with their baseline tests. Only one of six patients with clinical evidence of acute upper airway disease without evidence of lower airway involvement had a deterioration in pulmonary function. We found that the presence of elevated H_2O_2 in the expired breath condensate did not differentiate between acute upper and lower airway disease. While the asthmatic subjects as a whole had a mean expired breath H_2O_2 concentration that was elevated compared with controls (0.81 ± 0.70 versus 0.25 ± 0.27 μmol/L), this was largely due to the elevated levels of H_2O_2 in the asthmatic subjects with acute airway disease. The mean expired breath H_2O_2 of 0.54 ± 0.56 μmol/L from the asthmatic subjects without acute airway disease was not different from that of control subjects. The mean value of 1.5 ± 0.50 μmol/L in asthmatics with acute airway disease was elevated, compared with both controls and asthmatic subjects without acute disease. The finding that H_2O_2 concentration does not discriminate between different locations of inflammation within the respiratory system is consistent with the results of other reports of elevated H_2O_2 in expired breath condensate that has been demonstrated in intubated adult patients with adult respiratory distress syndrome or acute focal infiltrates (5) and, using a sensitive chemiluminescent technique, in volunteers after breathing 100% oxygen or after cigarette smoking (14). Because expired breath H_2O_2 lacks specificity for a site of inflammation within the airway, clinical evaluation or possibly a method to separate air expired from the upper and lower airway could be used to determine the location of inflammation. Additionally, we found that the use of anti-inflammatory agents does not hinder the detection of increased H_2O_2 in expired breath in patients with sufficient airway inflammation in either the upper or the lower airway to result in symptomatology.

The correlation between expired breath H_2O_2 concentrations and pulmonary function test results was not statistically significant when all evaluations were considered. This lack of significant correlation could be due to characteristics of two patient subgroups. First, those with clinical evidence of acute upper airway disease may have had inflammation and increased H_2O_2 production from the upper airways but without extension of the inflammatory process to the lower airways, and thus had no evidence of alteration in pulmonary function. Second, patients who had previously experienced an acute episode of airway inflammation that had since resolved and thus had a low H_2O_2 concentration may have had residual abnormal airway obstruction that persisted well past the inciting event (15). If the correlation is reevaluated excluding the first group of subjects (table 4; numbers 9b, 10b, 14, 15a, 16, and 20a), there is a significant correlation between H_2O_2 concentration and pulmonary function test result ($p = 0.01$). If the second group of subjects is excluded (table 4; numbers 3b, 5b, 9a, 10, and 19), the correlation between H_2O_2 concentration and decrease in pulmonary function still does not achieve statistical significance ($p = 0.20$). If both groups are excluded, there is a significant correlation ($p = 0.007$). Therefore, it appears that both patient subgroups may contribute to the lack of significant correlation between decreased pulmonary function test values and H_2O_2 concentrations in expired breath condensate, but the major discrepancy occurs in patients with upper airway inflammation without lower respiratory tract involvement.

A factor that could potentially lead to a falsely elevated expired breath H_2O_2 concentration could be contamination of the sample with saliva, which has a relatively high level of H_2O_2. We gave great care to using an effective saliva trap within our collection system to minimize this possibility. Of the 25 evaluations of asthmatic subjects who were clinically well, there were only two samples with an expired breath $H_2O_2 > 1.0$ μmol/L (2.1 and 2.5 μmol/L). In each of these cases, the subject had abnormal pulmonary function test results. The concentration of expired breath H_2O_2 of 0.25 ± 0.27 μmol/L in our control subjects was significantly lower than a previously reported level of 2.4 ± 1.7 μmol/L ($n = 10$) in nonintubated adult volunteers (5), but was similar to the control values for intubated adults of 0.34 ± 0.08 μmol/L ($n = 27$) reported by Baldwin and colleagues (4) and 0.99 ± 0.72 μmol/L ($n = 22$) reported by Snzajder and associates (5). These results indicate that saliva contamination was not a confounding factor in our expired breath samples.

Factors related to the asthmatic process itself may affect the ability to detect airway inflammation by evaluation of H_2O_2 concentrations in expired breath. Alterations in the respiratory pattern that would increase the exposure of air containing H_2O_2 from an area of inflammation to air with minimal H_2O_2 content would reduce H_2O_2 detection even in the face of increased production. Sznajder and associates (5) found that variations in minute ventilation did not affect the expired breath H_2O_2 in their group of intubated adult patients and in an animal model. Due to variations in airway hyperresponsiveness, some asthmatics may have abnormal pulmonary function related to relatively mild increased airway inflammation that cannot be differentiated from the range of normal values. Several factors, such as exposure to oxidants (16) and exposure to inflammatory mediators (17, 18), may induce increased antioxidant defenses. In patients with chronic inflammation, increased H_2O_2 production may be undetected by evaluation of expired breath because of increased scavenging. Although these factors did not hinder our ability to detect increased expired breath H_2O_2 in asthmatics with clinical evidence of acute respiratory disease, they are potential problems that could be encountered in investigations of more subtle alterations in respiratory tract inflammation.

Acute inflammation at any site within the respiratory tract may lead to an elevated concentration of expired breath H_2O_2. It is unlikely that the association of expired breath H_2O_2 with airway inflammation applies only to asthmatic patients. Moreover, other pathologic processes involving acute inflammation of the respiratory system may have the same association. Other information is required to determine the site of acute inflammation within the respiratory tract, and further investigation is needed to support specific application of this technique to a given pulmonary disorder. Our study demonstrates that evaluation of expired breath con-

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densate H_2O_2 concentration is a simple, noninvasive technique that may be utilized as a marker of acute airway inflammation.

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